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Successful desensitization with proteasome inhibition and costimulation blockade in sensitized nonhuman primates

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Key Points

- Targeting both PCs and GC response reduces donor-specific antibodies and prolongs graft survival in sensitized NHP kidney transplantation.

The detrimental effects of donor-directed antibodies in sensitized transplant patients remain a difficult immunologic barrier to successful organ transplantation. Antibody removal is often followed by rebound. Proteasome inhibitors (PIs) deplete antibody-producing plasma cells (PCs) but have shown marginal benefit for desensitization. In an allosensitized nonhuman primate (NHP) model, we observed increased germinal center (GC) formation after PI monotherapy, suggesting a compensatory PC repopulation mediated via GC activation. Here we show that costimulation blockade (CoB) targets GC follicular helper T (Tfh) cells in allosensitized NHPs. Combined PI and CoB significantly reduces bone marrow PCs (CD19–CD20–CD38+), Tfh cells (CD4+ICOS–PD-1hi), and GC B cells (BCL-6–CD20–); controls the homeostatic GC response to PC depletion; and sustains alloantibody decline. Importantly, dual PC and CoB therapy prolongs rejection-free graft survival in major histocompatibility complex incompatible kidney transplantation without alloantibody rebound. Our study illustrates a translatable desensitization method and provides mechanistic insight into maintenance of alloantibody sensitization.

Introduction

Kidney transplantation is the preferred treatment of end-stage renal disease with improved patient survival and quality of life compared with dialysis.1,2 However, patients with preformed donor HLA-specific antibodies (DSA) are more difficult to transplant because they require a stringent HLA match for a compatible donor kidney.3 Desensitization treatments reduce DSA in these patients to increase the pool of suitable donors. Desensitization therapies have been limited to combinations of plasmapheresis and IV immunoglobulin.4 Pretransplant, these treatments allow for successful implantation without hyperacute rejection, and posttransplant, they reduce the risk of antibody-mediated rejection5 as a result of antibody rebound.6,7 Desensitization treatments have been most successful in patients with an incompatible living donor; sensitized patients awaiting a compatible deceased donor transplant often have a prolonged wait to obtain a transplant8 and face reduced patient survival.5

It has been suggested that plasma cells (PCs), which are not targeted directly by current desensitization methods, contribute to the rebound in humoral responses seen after desensitization.9,10 Rituximab, a CD20-specific monoclonal antibody (mAb), has also been added to desensitization regimens to deplete B cells, with the hope of reducing PC generation and subsequent antibody production.11

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Figure 1. Dual targeting with proteasome inhibitor and CoB (belatacept and anti-CD40 mAb [2C10]) successfully promoted desensitization. (A) Timing and dosing of bortezomib, belatacept, and anti-CD40 mAb's for desensitization and biopsy scheme for treated animals. (B) DSA from T-cell flow-cytometric cross-matching of sensitized animals before and after dual targeting treatment. DSA levels are expressed as mean channel fluorescent intensity (MFI) ratio. Serum DSA level was significantly reduced after CoB treatment (pre- vs posttreatment). (C) Visualization of BM PCs. Dual targeting treatment significantly affected CD19<sup>+</sup>CD20<sup>-</sup>CD38<sup>+</sup> cells in the BM biopsy after dual targeting treatment. Representative flow plot (CD20 gated) and percentage of the CXCR5<sup>+</sup> BM plasma cells. (D) Tfh cells were traced with PD-1 and inducible T-cell costimulator (ICOS) from the LN biopsies. LN-Tfh cells showed a significant reduction after dual targeting treatment. Representative flow plot (CD20 gated) and percentage of the CD19<sup>+</sup>CD20<sup>-</sup>CD38<sup>+</sup> B cells in the LN biopsy after CoB treatment. Representative flow plot (CD20 gated) and percentage of the CXCR5<sup>+</sup> BCL-6<sup>+</sup> GC B-cell population in the LN. (E) A strong trend of reduction of CXCR5<sup>+</sup> BCL-6<sup>+</sup> cell population in the LN. (F) Proliferating IgG B cells (Ki67<sup>+</sup>IgG<sup>+</sup>IgD<sup>+</sup>CD20<sup>+</sup>) and Isotype switched B cell proliferation in the BM at the indicated time points (pre- vs posttreatment). (G) Immunofluorescent analysis of LN including B-cell follicles and GC staining for Ki67 (green), CD20 (red), and CD3 (blue). Original magnification ×200. Quantification of positive fluorescence signal of CD20 for B-cell follicle, and Ki67/CD20 for proliferating GC. Data represent the mean ± standard deviation (SD) of 4 monkeys per group. NS, nonsignificant.
However, B cells lose expression of CD20 upon terminal differentiation to PCs; consequently, rituximab conveys very limited efficacy in depleting PCs. More recently, proteasome inhibition (PI) targeting PCs was tested in desensitization protocols but has shown marginal benefit. We have previously demonstrated that PI with bortezomib for desensitization depleted PCs but did not reduce levels of DSA, possibly because of compensatory upstream germinal center (GC) expansion. In the present study, we demonstrate that targeting both PCs and follicular helper T (Tfh) cells successfully reduces DSA and
transplants in these pairs were performed as previously described.17
received no treatment prior to kidney transplantation. Renal
therapy, which was required in previously reported immunosup-
prolongs rejection-free graft survival in presensitized nonhuman
primate (NHP) kidney transplantation.

**Methods**

Male, outbred rhesus macaques (Macaca mulatta) were housed in
Yerkes National Primate Research Center (Atlanta, GA) or Duke
Laboratory Animal Resources (Durham, NC). Donor-recipient pairs
were selected based on full major histocompatibility complex class I
and maximal major histocompatibility complex class II mismatches
by 454 sequencing (supplemental Figure 1). All experiments were
compliant with the Emory and Duke Institutional Animal Care and
Use Committee. Skin grafts (~2.5 cm diameter) were swapped
between paired animals for allosensitization. Sensitized animals
were treated with belatacept (20 mg/kg), anti-CD40 mAb (2C10; 20
mg/kg), and bortezomib (1.3 mg/m²) or carfilzomib (20 mg/m²)
twice weekly, IV over 4 weeks for desensitization. Control animals
received no treatment prior to kidney transplantation. Renal
transplants in these pairs were performed as previously described.17
All recipients received induction with 0.3 mg/kg basiliximab IV on
postoperative day (POD) 0 and 4; 0.05 mg/kg tacrolimus intramus-
cularly twice daily (target trough: 8-12 ng/mL); 15 mg/kg mycophene-
nolate mofetil (MMF) subcutaneously or 30 mg/kg MMF orally; and
125 mg methylprednisolone IV (tapered daily). Peripheral blood,
lymph node (LN), and bone marrow (BM) cells were processed and
stained with fluorochrome-conjugated antibodies as described in
the supplemental Methods. Pathological evaluation was performed
by a pathologist (A.B,F,) according to the updated Banff 2007
criteria.18,19 Kaplan-Meier and log-rank tests were used to compare
graft survival. Sample comparisons of same animals were achieved
by paired t test and Student t test for others. P < .05 was considered
statistically significant.

**Results and discussion**

CD28 and CD40 expression on multiple myeloma cells and long-
lived PCs has been documented.20-22 Therefore, we hypothesized
that targeting PCs with a B7 costimulatory molecule-specific fusion
protein (belatacept, Bristol Myers Squibb) and a CD40-specific
mAb (2C10, Mass Biologics) could interfere with PC homeostasis
and limit PC function. However, we found that DSA level and BM
PCs were not significantly affected by combined costimulation
blockade (CoB) treatment. Nevertheless, significant reductions in
Tfh and GC-B cells, and reduced isotype switched B-cell
proliferation, were observed in LNs (supplemental Figure 2). These
data suggest that targeting both B7/CD28 and CD40/CD154
signaling does not suppress BM PCs but significantly reduces Tfh
cells in the sensitized host. Therefore, we hypothesized that the
effect of PI with bortezomib to deplete preformed PCs, when
combined with CoB using belatacept and 2C10, would be
synergistic,16 controlling both critical T- and B-cell interactions for
PC regeneration, avoiding post-PC depletion homeostatic activation,
and resulting in desensitization of sensitized NHPs (Figure 1A).
We found that this “dual targeting” strategy significantly reduced
DSA levels over 4 weeks in sensitized NHPs (Figure 1A). Tfh, GC-B,
and proliferating B cells in LNs were also reduced after treatment (Figure 1D-F). We performed in situ GC
staining to confirm the attenuated GC response after dual targeting.
The average B-cell follicle size (CD20 area per follicle number per LN)
was not significantly different. However, animals treated with dual
targeting showed less frequent GC containing follicles and
significantly reduced GC size (Ki67+CD20+ area per follicle area)
(Figure 1G). Interestingly, the CD4+ Tcm cell levels declined after
desensitization (supplemental Figure 3). These data show that dual
targeting with CoB and PI modified not only the B-cell and PC
compartment but also T-cell components.

To evaluate the durability of dual targeting desensitization and its
application to solid organ transplantation, we performed kidney
transplantation after treatment and compared graft survival with
nondesensitized controls. Five control animals received kidney
transplantation from their previous skin donors. As shown in
Figure 2A, 3 animals were treated twice weekly with bortezomib or
carfilzomib and belatacept and 2C10 for 1 month before
transplantation. Controls and desensitized animals received basili-
imab induction with conventional maintenance immunosuppression
(tacrolimus, MMF, and steroids). Sensitized animals showed
accelerated rejection with mean survival time (MST) of 3.6 days,
whereas sensitized animals treated with dual targeting therapy
pretreatment had prolonged MST (Figure 2B; MST >58.6 days, P < .05). Two animals treated with bortezomib were euthanized
because of weight loss with normal serum creatinine. Carfilzomib
was later substituted for bortezomib in 1 subject because of
bortezomib-associated weight loss. Although this subject showed
transient posttransplant weight loss, at 6 weeks posttransplantation
the weight was regained, and prolonged graft survival was observed
(supplemental Figure 4). This animal was euthanized at POD 120
with normal graft function. No early graft injury or rejection was
observed in biopsies from monkeys desensitized with dual targeting
(data not shown) despite the lack of T-cell–depleting induction
therapy, which was required in previously reported immunosup-
pressive protocols to avoid rejection.17 Allograft histology was
evaluated at necropsy. Desensitized animals did not show evidence
of rejection in contrast to sensitized controls (Figure 2C). Sensitized
controls showed profound infiltration of T and B cells and
macrophages in the grafts at early time points without desensiti-
zezation, whereas less infiltration was observed in desensitized
animals at later time points (Figure 2D). No significant increases
in posttransplant DSA levels, GC responses, or memory T cells
were observed in long-term recipients, suggesting a durable effect
of dual targeting desensitization (Figure 2E-F). This reflects another
missing concept in current desensitization approaches, namely, the
need for targeting the reemerging antidonor response. This concept
is not limited to Pts and CoBs but extends to agents targeting
the effector arm of the humoral response in combination with
influencing T-cell help for antidonor B-cell responses.

These results suggest that dual targeting of PC and GC profoundly
alters alloimmunity in sensitized hosts, permitting long-term graft
survival and preventing alloantibody rebound, which illustrates the
potential of this strategy for treating HLA-sensitized humans and
antibody-mediated rejection.

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Authorship

Contribution: J.K. designed experiments, performed surgical procedures and cared for experimental macaques, interpreted data, and prepared the manuscript; C.B. designed experiments, performed surgical procedures and cared for experimental macaques, and conducted in vitro experiments; M.M., B.E., and J.P. participated in surgical procedures and cared for experimental macaques, and prepared the manuscript; J.Y. processed tissue samples, conducted in vitro experiments and performed flow cytometry; J.S.Y. performed flow cytometry; N.I. participated in initial experimental design; A.G. conducted in vitro experiments and performed flow cytometry; J.J.H. performed immunohistochemistry; A.B.F. interpreted data (pathologist); A.D.K. interpreted data and prepared the manuscript; and S.J.K. conceived of experimental design, performed surgical procedures, cared for experimental macaques, interpreted data, and prepared the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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